

REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-4, 6, 8-11, 13-16, 19-23 and 26- 30 are in this case. Claims 1-4, 6, 8-11, 13-16, 19-23 and 26- 30 have been rejected. Claims 6 and 19 have now been cancelled. Claims 1, 3, 14, 15, 16, 21 and 22 have now been amended.

Claim Objections

The Examiner has objected to claim 3 due to a misspelling of "Agrobacterium" in line 3. Claim 3 has now been corrected, the term "Agrobacterium" correctly spelled.

Drawings

The Examiner has objected to the drawings. Corrected formal drawings are respectfully submitted herewith.

35 U.S.C. § 112, First Paragraph, Rejections

The Examiner has rejected claims 1-4, 6-11, 13-16, 19-23 and 26- 30 under U.S.C. § 112, first paragraph, as containing subject matter that was not described in specification in such a manner as to enable one skilled in the art to make and/or use the invention commensurate in scope with these claims. The Examiner's rejections are respectfully traversed. Claims 6 and 19 have now been cancelled, rendering moot the Examiner's rejections thereof. Claims 1, 3, 14, 15, 16, 21 and 22 have now been amended. The Examiner states that the instant specification and the claims as originally filed lack support for the phrases "a polynucleotide capable of hybridizing under high stringency conditions" and "wherein said polynucleotide encodes a polypeptide having organic carbon fixation activity". Contrary to the Examiner's contention, it is the Applicant's strong opinion that the instant specification and original claims provide support for the abovementioned phrases.

The abovementioned notwithstanding, in order to expedite the prosecution, the Applicant has elected to delete the phrase "hybridization under high stringency conditions..." recited in claims 1 and 16, and to replace it with the phrase "...encoding

a polypeptide having an amino acid sequence at least 95% homologous to the sequence as set forth in SEQ ID NO:3..." as recommended by the Examiner in the telephone interview of July 24, 2003. Thus, now amended claims 1 and 16, and all claims depending therefrom, include the limitations of now cancelled claims 6 and 19, defining the polynucleotide of the "nucleic acid construct" in terms of strict sequence homology. Support for such an amendment is found throughout the instant specification (see, for example, description of sequence analysis, page 50, line 14 to page 51, line 19; and the alignment of homologous amino acid sequences derived from ORFs of nucleic acid sequences on pages 52 to 54; see, also, now cancelled claims 6 and 19).

The Examiner has stated that the phrase "a polypeptide having an inorganic carbon fixation activity" is not supported in the instant specification. In accordance with the Examiner's recommendation, expressed during the telephone interview which took place on Thursday, July 24, 2003, reference to "a polypeptide having an inorganic carbon fixation activity" has been removed from now amended claims 1 and 16, and has been replaced with the limitation: "a polypeptide having a bicarbonate transporter activity", as recited in the claims as originally filed.

The Examiner has requested clarification regarding the relevance of the reference cited in the previous communication of May 28, 2002 (Omata et al, PNAS 1999, 96:13571-76), from which the Examiner concluded that the "enzymatic activity of the gene of SEQ ID NO:2 (*ictB* gene) remains in question". Such clarification is provided hereinbelow, and further in the enclosed Declaration by Prof Aaron Kaplan.

Firstly, regarding the role of bicarbonate transporters in CO_2 acquisition systems, Applicant wishes to point out that the ability to actively concentrate CO_2 , against a gradient, despite the low affinity of Rubisco for CO_2 , is well known to result from the activity of at least 4 separate enzymatic systems. As has been recently summarized in a review by Ogawa and Kaplan (Photosynthesis Research, in press, preprint enclosed), two CO_2 uptake systems (an induced and a constitutive system), and two HCO_3^- transporter systems, the *cmpA-D* system, and the *Sbt-A* are active in cyanobacteria (see Table 2, page 4). Furthermore, mutations induced in the *cmpA-D* system in *S. 6803* had hardly any effect on HCO_3^- transport activity and no effect on growth characteristics, compared to wild type plants (see page 4, left column,

paragraph 1), indicating the limited role, if any at all, of the *cmpA*-D system in Ci acquisition.

Thus, it is evident that the *cmpA*-D HCO_3^- transporter system plays only a minor role in *S. 6803* growth, and that other, more important HCO_3^- transporter systems operate in this, and other, photosynthetic organisms. Indeed, the Applicants have recently shown that double mutation in a *S. 6803* strain causing inactivated CO_2 uptake and sodium dependent bicarbonate transport (*sbtA*) HCO_3^- uptake results in a loss of ability to grow under low CO_2 conditions despite the fact that *cmpA*-D activity remained unaffected (Shibata et al, *J Biol Chem* 2002, 277:18658-64, enclosed; see Figs. 1A and 1B).

Further, Applicant wishes to point out that recent, unpublished results in his laboratory demonstrate that *Synechocystis* 6803 mutants deficient in both CO_2 uptake and the *sbt-A* and the *cmpA*-D HCO_3^- uptake systems grow under conditions of limiting CO_2 when exposed to high salinity, due to activation of the *ictB* system. Thus, the existence of multiple pathways for Ci acquisition, and the critical role for the *ictB* bicarbonate transporter in photosynthetic plants, is clearly demonstrated, emphasizing the fallacy of Omata's conclusions. Indeed, the evidence for multiple pathways for Ci acquisition is discussed in the instant specification (page 53, line 15 to page 54, line 1).

Further, the Examiner has stated that "enzymatic activity of the gene of SEQ ID NO:2 (*ictB* gene) remains in question". It is applicant's strong opinion that the bicarbonate transporter activity of the *ictB* gene product is amply demonstrated in the instant specification. For example, *Synechococcus* PCC 7942 cyanobacteria having inactivated *ictB* (IL-2) were severely deficient in CO_2 uptake, compared with wild type (Figure 4a and 4b). The impaired HCO_3^- uptake was especially notable in low CO_2 concentration (see Table 1, page 47). In another example, transgenic plants expressing the *ictB* gene demonstrated superior photosynthetic rate, compared to wild type plants, under conditions of limiting CO_2 concentration, such as low humidity and low CO_2 tension (see Table 2, page 55). Further evidence of the bicarbonate transporter activity of the *ictB* gene product is provided by recent studies by the

Applicant, demonstrating enhancement of bicarbonate transport, resulting in increased inorganic carbon fixation by transgenic tobacco plants expressing the *ictB* gene (see Appendix IV, Figure 12, enclosed herein). Briefly, RubisCO activity was measured in wild type, and transgenic tobacco plants expressing the *ictB* gene, under conditions of low humidity (stomatal closure, limited gas exchange), and thus, only partial CO₂ saturation of the enzyme complex. RubisCO activity was expressed as rate of carboxylation, directly measuring nmol CO₂ fixed per nmol active sites. The transgenic plants (open circles) clearly had superior carboxylation rates under non-activated conditions (open circles) compared to the wild type controls (open squares). That this superior inorganic carbon fixation was due to an increased availability of CO₂ substrate, and not to alteration of Rubisco catalytic properties, is demonstrated by the kinetics (S/V vs. S) plots in the inset: note the higher reaction rate (V max) but similar substrate affinity (K_m) of the Rubisco activity in transgenic and wild type plants. Thus, the expression of the *ictB* gene in the transgenic tobacco plants resulted in increased CO₂ availability under conditions of HCO₃⁻ transport dependent Ci acquisition. Further strong evidence that the internal CO₂ concentration, at the site of CO₂ fixation, was higher in the transgenic *Arabidopsis* and tobacco plants expressing *ictB* is provided by the observation of lowered CO₂ compensation point in the transgenic plants as compared with the wild types (see Lieman-Hurwitz et al, Plant Biotech Journal 2003;1:43-50, Table 2, enclosed herein).

Thus, it is Applicant's strong opinion that the nucleic acid construct recited in now amended claims 1 and 16, and claims directly or indirectly dependent therefrom, now comprises a polynucleotide clearly defined by both physical ("polynucleotide encoding a polypeptide having an amino acid sequence at least 95% homologous to the sequence as set forth in SEQ ID NO:3...") and functional ("encoding a polypeptide having a bicarbonate transporter activity") criteria. In view of the abovementioned arguments and amendments, Applicant believes to have overcome the rejection based on introduction of NEW MATTER.

The Examiner has further rejected claims 1-4, 6-11, 13-16, 19-23 and 26-30, stating that the specification fails to provide guidance for a nucleic acid that hybridizes to SEQ ID NO:2 and that encodes a protein with inorganic carbon fixation activity, methods of using it, and plants thereby obtained. The Examiner further

states that identifying nucleic acids functionally related to a given nucleic acid is highly unpredictable, and that a great many proteins have “inorganic carbon fixation activity”, requiring “undue trial and error experimentation of one of ordinary skill in the art.

The Examiner has also stated that the specification fails to provide adequate description of the claimed invention, since the “claims are broadly drawn to a multitude of DNA molecules that hybridize to SEQ ID NO:2, or that comprise “any variation of a portion of any size of SEQ ID NO:2...and the specification only describes a nucleic acid from *Synechococcus* that comprises SEQ ID NO:2”.

Applicant wishes to point out that the restrictions imposed by now amended independent claims 1 and 16, namely, a “...polynucleotide encoding a polypeptide...having an amino acid sequence at least 95% homologous to the sequence as set forth in SEQ ID NO:3...” and a “polynucleotide encoding a polypeptide having a bicarbonate transporter activity...” constitute clear criteria by which candidate polynucleotides can be screened. Indeed, using the methodology described in the instant specification and in the Response to Official Action filed by the Applicant on November 29, 2002, for identifying sequences homologous to the *ictB* coding sequence (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:3), the Applicants have recently succeeded in identifying a number of highly conserved peptide domains (see hydropathy plots, Appendix I and II, Fig 10a and 10b, enclosed herein) which are characteristic of the *ictB* protein and its homologues from other species (see Appendix III, Fig 11, amino acid sequence alignment, enclosed herein).

Briefly, the *IctB* protein from *Synechococcus* PCC 7942 and homologous protein *Synwh0268* from *Synechococcus* sp Strain WH 8102 were analyzed for characteristic transmembrane (hydrophobic) and hydrophilic domains using the TopPred program. Identification of proteins having significant homology, and alignment of the amino acid sequences was performed using the CLUSTALW multiple alignment program.

Thus, in view of the abovementioned amendments and new data detailed hereinabove, it is Applicant’s strong opinion that, provided the teachings of the present invention, one of ordinary skill in the art would be expected to be able to

make and use the nucleic acid constructs and selection methods disclosed therein without undue experimentation, and with a reasonable expectation of success.

35 U.S.C. § 112, Second Paragraph, Rejections

The Examiner has rejected claim 1-4, 6, 8-11, 13-16, 19-23 and 26-30 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that Applicant regards as the invention. Claims 6 and 19 have now been cancelled, rendering moot the Examiner's rejection thereof. Claims 1, 14, 15, 16, 21 and 22 have now been amended. The Examiner's rejections are respectfully traversed.

The Examiner has stated that the phrase "further includes", in claim 15, lacks antecedent basis in independent claim 1. Claim 15 has now been amended, according to Examiner's recommendations, to now recite "wherein said polynucleotide further comprises:" (emphasis added). Claim 1 has now been recommended to recite: "...nucleic acid construct comprising...", providing antecedent basis for the recitation of "further comprising" of claim 15.

The word "derived" has now been deleted from now amended claims 15 and 22.

The word "independently" has been deleted from now amended claims 14 and 21.

The phrase "being for directing nuclear transcription..." in now amended claim 16 has been replaced with the phrase "capable of directing nuclear transcription...".

Regarding the Examiner's rejections of claims 1 and 16 as indefinite in the recitation of "high stringency conditions", and "inorganic carbon fixation activity", claims 1 and 16 have now been amended to recite:

"...a polynucleotide encoding a polypeptide having a bicarbonate transporter activity, wherein said polypeptide is at least 95% homologous to SEQ ID NO: 3, as determined using Blast software where gap open penalty equals 11, gap extension penalty equals 1 and matrix is blosum 62..."

Applicant's arguments and description of support for such amendments are described in detail hereinabove.

In view of these amendments Applicant believes to have overcome the 35 U.S.C. § 112, second paragraph rejections.

35 U.S.C. § 102 (b) Rejections- Ko, et al.

The Examiner has rejected claims 1-4, 8-9, 13-16, 20-23, 26-27 and 30 under 35 U.S.C. § 102 (b) as being anticipated by Ko et al. Claims 1, 3, 14, 15, 16, 21 and 16 have now been amended. The Examiner's rejections are respectfully traversed.

The Examiner states that Ko et al. discloses a nucleic acid that would hybridize under unspecified "high stringency conditions" to SEQ ID NO:2.

Independent claims 1 and 16 have been amended, no longer reciting "high stringency conditions", but rather:

"...a polynucleotide encoding a polypeptide having a bicarbonate transporter activity, wherein said polypeptide is at least 95% homologous to SEQ ID NO: 3, as determined using Blast software where gap open penalty equals 11, gap extension penalty equals 1 and matrix is blosum 62...".

Thus, the polynucleotides of the present invention are clearly defined by criteria of functionality and sequence homology.

Applicant wishes to point out that the polynucleotides taught in Ko et al. encode the chlorophyll a/b binding protein Cab-2. BLAST analysis of the sequence, and related homologues, reveals no homology between the Cab and ictB nucleotide sequences (see enclosed BLAST results). Thus, the methods and nucleic acid constructs taught by Ko et al. do not anticipate the nucleic acid constructs and methods for obtaining photosynthetic plants characterized by enhanced inorganic carbon fixation.

35 U.S.C. § 103 (a) Rejections- Ko, et al. in view of Gordon Kamm et al

The Examiner has rejected claims 1-4, 8-11, 13-16, 20-23, and 26- 30 under 35 U.S.C. § 103 (a) as being anticipated by Ko et al. in view of Gordon Kamm et al. Claims 6 and 19 have now been cancelled, rendering moot the Examiner's rejections thereof. Claims 1, 3, 14, 15, 16, 21 and 22 have now been amended. The Examiner's rejections are respectfully traversed.

Applicant wishes to point out that the polynucleotides taught in Ko et al. encode the chlorophyll a/b binding protein Cab-2. BLAST analysis of the sequence, and related homologues, reveals no homology between the Cab and ictB nucleotide sequences (see enclosed BLAST results). Thus, the methods and nucleic acid constructs taught by Ko et al. do not anticipate the claimed nucleic acid constructs and methods for obtaining photosynthetic plants characterized by enhanced inorganic carbon fixation.

In view of the above amendments and remarks it is respectfully submitted that claims 1-4, 8-11, 13-16, 20-23 and 26-30 are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,



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Date: August 8, 2003.

Encl.:

Corrected formal drawings
A two months extension fee
BLAST analysis Cab-2 vs ictB
Appendix I-IV: Figures 10, 11 and 12
References:

Ogawa and Kaplan (Photosynthesis Research, in press)
Shibata et al, J Biol Chem 2002, 277:18658-64
Lieman-Hurwitz J et al. Plant Biotech Journal 2003;1:43-50

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ICTB : 1 ATGACTGTCTGGCAAACACTCTGACTTTGCCATTACCAACCCAAACAGTGGGCCACAGC 60 (SEQ ID NO: 2)
SLR : 13 ATCTCTATCTGGGATCGCTGATGTTGGGGTTCCAGGAATGGGGGGGC 72 (SEQ ID NO: 4)

ICTB : 61 AGTTTCTGCATCGGCTGTTGGCAGCCTGC-GAGCTTGGCCGGCCTCCAGCCAGCTGTT 119
SLR : 73 AGTGTGCTCCATCGTTGGGGCTGGGACAGAG-TGGATAACGGCTAGTGTGCTCTG 131

ICTB : 120 GGTTGGTCTGAGGCCACTGGGT--GGCTTCTTGCTGCTCTACGGTTCGGCTCCG 177
SLR : 132 GCCCACTTCAGGGCATGGTACGGCT-CTAG-TGGCAATAATTTTTATTGGCTCCC 189

ICTB : 178 TTGTGCCAGTCCGGCTAGGGTTGGGCTAGCCGATCGCG-GCCTATTGGCCCT 236
SLR : 190 TTCACTCCACCATGTTGGCATTTTAT-GCTGCTCTGTGGAGCCTTTGGCTCT 248

ICTB : 237 GCTCTGGCTGACAGATACTGATCTGCGAAGCA---ACCCCCATTCACTGGCTGGCT 293
SLR : 249 GCTGACCTTGGCTGAT--CAACCAAG-GGAAGGGTTGACTCCCATGTTTAGTTT 305

ICTB : 294 GCTCTACTGGGGCTGATGCCCTAGCAAACGGGACTCTCACCCGTACGGCTGCAGCTTT 353
SLR : 306 TGCCCTACTGGTGCATTGGGATTCGGCTTCAAGCTAAATTAGCTTAC-TGGGGGGGGC 365

ICTB : 354 AGTTGGGCTAGCCAAACTGAC-GCTC-TACCTGTTGGCTTGGCCCTAGGGCTGGGTT 411
SLR : 366 GTGGGGTTAGCCGAATTAAACAGCTAAATTATGTTAGTGTCTAC-TGGGGGGCTATT 423

ICTB : 412 CTCCGCAATCCCCGCTGC-GATCGCTGCTGGTCTCGGTGATCACATCGCTTT 470
SLR : 424 TTGCAAAACAAATGGTGAAC-CGGTTAGTAACCGTGTAGTTACTGGTAGGGCTATT 482

Fig. 2A

Fig. 2b

Fig. 2c

Fig. 2

Fig. 2d



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Fig. 26 (Continued)

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ICTB : 943 RACTTCCGGATAATGTCCTGGCGGTGCTGCAGATGATTCAAGATCGGCCCTGGCTG 1002
SLR : 955 AATTTCGGCATCAATGTCATGTTGGAAAGGGTAAAGCCATGATCCGAGCCCCTATCATT 1014

ICTB : 1003 GGCAATGGCCCCGGCAATAACCGCCTTAACCTGGTTTAATCCCTCTATCAACAGGGGCCGC 1062
SLR : 1015 GGCAATGGCCCCAGGTAAACCAAAATTATCCTACTATATGGGGCCCC 1074

ICTB : 1063 TTTACGGCGTTGAGCGGCCCTACTCCGGTGGAAAGTCGCCGGTGGACTACTG 1122
SLR : 1075 TTCAACGGCCCTGAGTGCCCTATCCATTACCTAGAAATTGTTGGTGAACGGGTGTAGTT 1134

ICTB : 1123 GGCTTGA-CGGCCTTGGCTTGGCT-GCTGGTCAACGGGGTGACCGGGGTGGCAGG 1180
SLR : 1135 GGTTTACCTGTATGCTC-TGGCTGTTGGCTTACCCCTAGGCAAAGGC-GTAGAACTGG 1192

ICTB : 1181 TGAGCCGACTTGGCGGATCGCAATCCCC--AACGCCTTTGGTGTGGCTAGCTTGGC 1238
SLR : 1193 TAAACCG-CTGTCGC-CAAACCCCTGGGGATGGCATCTGGATTATGGGGCTTTAGC 1250

ICTB : 1239 CGGTGGCAGGAATGCTGGGTCAACGGTCTGTTGATAACCGGTCTATCGACCGGAAGC 1298
SLR : 1251 GGCGATCATGGTTGGTCCACGGCATGGTAGATACTGCTGTACCGTCCCCGGT 1310

ICTB : 1299 CAGTACGGCTCTGGTGGCTCTGTATTGG--AGCGATGGGAGTTCTGG--CAGC-CCCAA 1353
SLR : 1311 GAGGCACTTCTGGTGG-TCGCTAGTGGCCATTGCTAGTCAGTGGCTACGGGACAA 1368

ICTB : 1354 CCTTCCAAAGCAACTCCCTCCAGAACCGGAGCATTAGACGAA 1395
SLR : 1369 GCCCCTTGGAGGGCAAGTAAAGAA---GAAATGAGGACAAA 1407

Fig. 2C
(Continued)